No long lasting or intermittent mast cell activation in acute coronary syndromes


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Unstable coronary syndromes, such as acute myocardial infarction and unstable angina pectoris are mostly due to rupture of an atherosclerotic plaque. Recently mast cells were found to participate actively in the inflammatory process of atherosclerosis by excreting proteolytic and pro-inflammatory substances with the ability to cause plaque instability and rupture. Mast cell activity can be determined by measuring serum levels of tryptase, as has been demonstrated in patients with anaphylaxis and mastocytosis. We hypothesised that acute coronary events (acute myocardial infarction and unstable angina pectoris) are associated with elevated serum tryptase levels as a reflection of increased mast cell activity. Serum levels of tryptase were determined in the following three groups of patients: 13 patients with acute myocardial infarction, 10 patients with unstable angina pectoris, and 14 patients without ischaemic cardiovascular disease who were used as controls. Patients with known IgE mediated allergic diseases and/or anti-histaminical drugs were excluded. The groups were comparable for sex, blood pressure, smoking and cholesterol levels. The controls tended to be younger (P = 0.05). Levels of tryptase did not differ between patients with acute myocardial infarction (7.9 ±4.6 µg/L), unstable angina pectoris (6.0 ± 2.1 µg/L) or controls (6.9 ± 4.1 µg/L), nor could a relation with levels of C-reactive protein be demonstrated. We conclude that serum levels of tryptase are not elevated in patients with acute coronary syndromes. This implicates that increased mast cell activity, if any, in unstable coronary syndromes is not reflected systemically. Other, more specific methods will be needed to determine the activity of the mast cell in vivo.

INTRODUCTION

Acute coronary syndromes such as unstable angina pectoris and myocardial infarction are usually the consequence of a thrombosis superimposed on an eroded or ruptured atherosclerotic plaque [1]. The site for plaque disruption is usually the place where the lesion is growing, the so-called “shoulder region” of the plaque. This region has a high cellular component, mainly consisting of macrophage and T-lymphocytes [2]. Remarkably, the fact that considerable numbers of mast cells are present in these plaque regions as well, has received little attention [3;4]. This is even more surprising since mast cells contain a large number of granules containing many mediators that may be involved in plaque rupture. Among these mediators are the proteolytical enzymes chymase and tryptase [5;6]. These enzymes are capable of activating matrix metallo-proteinases (MMPs) [7-9], which are secreted in an inactive proform by smooth muscle cells and macrophages [10;11]. Activated MMPs degrade the extracellular matrix (ECM) of atherosclerotic plaques and are also capable of activating other MMPs. As the ECM is thought to contribute to the strength of the plaque, degradation by MMPs has been implicated in plaque rupture. Furthermore, the production of MMPs is stimulated by pro-inflammatory cytokines such as TNFα, which is known to be involved in atherogenesis [12;13]. Moreover, products released during matrix degradation, in turn, activate mast cells. Multiple studies support the view that mast cell activation could be linked with plaque rupture. Accumulation of mast cells and increased expression of MMPs in the shoulder region of the plaque has been reported [3;11;14]. It is unknown, whether the duration of the mast cell activation is largely restricted to unstable plaque or that it occurs more widespread. If mast cell activation is immediately followed by plaque rupture and the mast cell activation takes place in a substantial part of all mast cells in the body, mast cell degranulation may be reflected by an elevation of the plasma tryptase level in patients with unstable angina pectoris and acute myocardial infarction. This mast cell specific
enzyme appeared to be a reliable and sensitive marker for mast cell activity in patients with systemic anaphylaxis and mastocytosis [6;15]. To test this hypothesis, we measured the serum levels of tryptase in patients suffering from acute coronary events.

**MATERIALS AND METHODS**

**Patients**

In this pilot study, blood samples were taken from consecutive patients admitted to the hospital with an episode of chest pain in the preceding 24 hours suspect for acute myocardial infarction or unstable angina pectoris. Patients were included during a 2-month period. Patients were divided into three groups based on the eventual diagnosis. Group A consisted of patients who appeared to have chest pain of another origin than myocardial ischemia, group B were patients who had unstable angina pectoris and group C suffered from acute myocardial infarction. Of each patient the time lag between onset of complaints and hospital admission was reported. Excluded were patients with a history of one of the following conditions: COPD, asthma, atopic constituency, concurrent infection, inflammatory disease, immunological disorders and major surgery or trauma within the last three weeks. Patients using anti-histamines, (inhaled) corticosteroids and other immunosuppressive medications were also excluded. The use of non-steroid anti-inflammatory drugs was allowed. All patients gave written informed consent and the study was approved by the local medical ethical committee. Chest pain from non-cardiac origin was defined as pain without any sign of myocardial ischaemia, assessed by electrocardiography (ECG), an exercise test or coronary angiography if performed. All patients with a rise in creatine kinase (CK) levels above twice the upper normal level were excluded from this group (group A). Unstable angina pectoris was defined as one or more recent episodes of chest pain in rest and typical for angina pectoris, in combination with one of the following findings: ECG changes suspect for myocardial ischemia, a positive exercise test indicating myocardial ischemia, coronary angiography showing significant coronary artery stenosis (more than 70%) or development of an acute myocardial infarction after admission to the hospital. Patients with elevation of CK levels of more than twice the upper normal level were excluded from this group (group B). Acute myocardial infarction was defined as the presence of a recent episode of chest pain or collapse, ECG abnormalities suspect for myocardial infarction and a rise of CK/CK MB ratio > 8% or a rise in CK MB over 20 U/L.

**Blood collection**

Venous blood samples were collected immediately after admission to the hospital. They were stored at room temperature (RT) for a maximum of 8h, after which they were stored at 6°C for a maximum of 24h. Serum was then stored at -20°C for a maximum of 3 months. The determination of tryptase in serum is not influenced by storage of 48h at RT [16;17].

**Tryptase assays**

Tryptase levels were determined using the B12 assay [18]. The measurements were performed using Pharmacia UniCAP Tryptase reagentia and the Pharmacia Unicap100 analysis device (Pharmacia & Upjohn, Uppsala, Sweden). Reference values for healthy individuals are those reported by Pharmacia & Upjohn, showing a geometric mean level of 5.6 µg/l and an upper 95 percentile of 13.5 µg/l (129 apparently healthy children and adults).

**Table 2.** Time delay between onset of symptoms and blood sampling in minutes

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr</td>
<td>210</td>
<td>90</td>
<td>720</td>
</tr>
<tr>
<td>UAP</td>
<td>313</td>
<td>60</td>
<td>720</td>
</tr>
<tr>
<td>AMI</td>
<td>398</td>
<td>60</td>
<td>1380</td>
</tr>
</tbody>
</table>

UAP= unstable angina pectoris, AMI= acute myocardial infarctio
In our hands this assay is able to detect differences in systemic mast cell activation. We illustrated this by determining tryptase levels in a population of patients with systemic mastocytosis (n = 30) (46.8 µg/l (10.2-200.0), median (95% CI)) and in patients suspected for mastocytosis who had no elevated mast cell count in bone marrow specimens (n = 1511.0(2.9-28.1)).

*C-reactive protein assays*
Levels of CRP were determined using an in-house ELISA as described before [19].

*Statistics*
Patient characteristics and tryptase levels were compared between the three groups using a Kruskall/Wallis non-parametrical test. Correlations of tryptase levels and blood pressure, age, cholesterol levels and time lag to presentation was determined using a Pearson correlation test.

**RESULTS**
During the inclusion period of 2 months, 65 patients were admitted to the hospital because of complaints of chest pain. Twenty-eight of were excluded because they did not meet the inclusion criteria. Therefore, the total study population consisted of 37 subjects, 8 females and 29 males. The characteristics of these subjects are given in table 1. The three groups were comparable for sex, total cholesterol level and blood pressure. There was a difference between the groups with respect to age as the patients in group A tended to be younger than those in groups B and C (P=0.046).
No significant difference in levels of tryptase between the three groups was found (figure 1). Moreover, levels of tryptase were all within the normal range [18]. For all three groups together, no relationship was found between tryptase levels and age, sex, total cholesterol, blood pressure, presence of smoking. The mean time lag was highest in the group of patients with acute myocardial infarction, and lowest in the group of controls (table 2). No correlation of time lag and tryptase levels was found ($R = -0.277$, $P = \text{n.s.}$). Levels of CRP (figure 2.) did not differ between the three groups. No correlation between CRP and tryptase levels was detected. ($R = 0.138$, $P = \text{n.s.}$).

**DISCUSSION**

Although there is ample evidence for the presence of mast cell activity in atherosclerotic lesions, we were not able to detect an elevation of tryptase levels in patients with acute coronary syndromes (groups B and C) as compared to a control group (group A). There are several factors that can account for the absence of elevated tryptase levels. Only a small number of mast cells may have been activated. Although mast cell density tends to be increased in unstable plaques, their absolute number is low when compared to numbers of T-lymphocytes and macrophages in the same plaques [20].

However, tryptase levels in the blood of a small group of patients with unstable angina pectoris were recently found to be raised sixfold within 5 min after onset of spontaneous myocardial ischaemia and to be declined to base-line values within 15 min. Remarkably, myocardial ischaemia induced by ergonovine administration did
not result in tryptase elevation, suggesting a role for mast cell degranulation in unstable angina pectoris [21]. The duration of the tryptase elevation in this study was shorter than expected. Plasma half time for tryptase is about 2 h [15]. Therefore, the findings of Cuculo et al. [21] need to be confirmed. It is possible that the absence of tryptase elevation in serum in our study was due to the time lag between the onset of chest pain and the moment of blood sampling. However, time lag and tryptase levels were not correlated.

Levels of C-reactive protein were determined to study a possible relation between tryptase levels and inflammatory activity in our patients. No correlation between levels of CRP and tryptase could be found in the patients suffering from acute coronary syndromes. A power analysis, based on the differences in tryptase levels between controls and the patients with AMI (6.9 ± 4.0 vs. 7.9 ± 4.6), was done. Given a power of 80 % and a type I error of 0.05, a total number of 600 patients will be needed to reach statistical significance. Since the differences between the groups were small, within the normal range, and the calculated sample size was large, we feel that further studies would not lead to clinically relevant results.

We conclude that a prolonged or frequent intermittent activation of a substantial part of mast cells is not likely to occur during unstable angina pectoris and acute myocardial infarction.

REFERENCES
Mast cell activation in acute coronary syndromes

2. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994; 89:36-44.


